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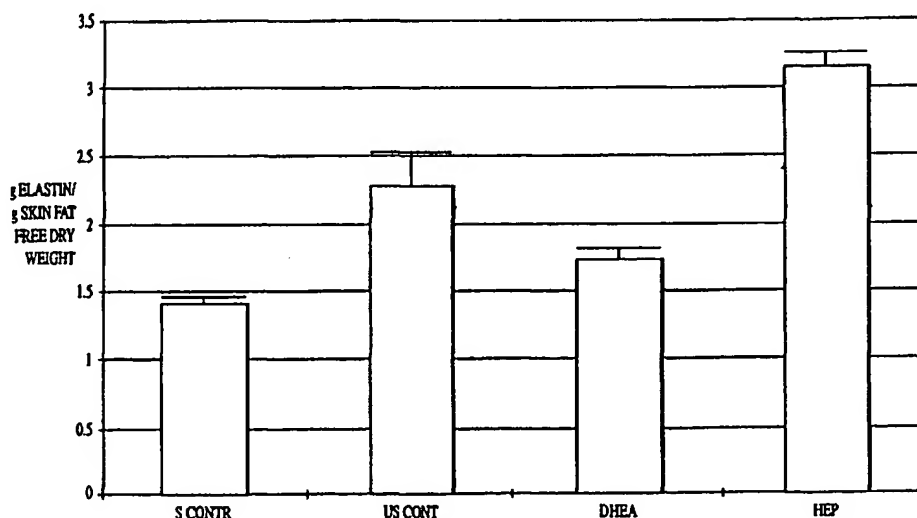
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(54) Title: **COMPOSITION AND METHOD FOR ENHANCING ELASTICITY OF TISSUE**



(57) Abstract: The present invention is directed to a composition and method used to enhance the elasticity and/or appearance of tissue. Specifically, the present invention is directed to a composition formulated from peptides having low molecular weights and which substantially correspond to sequences found in elastin.

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COMPOSITION AND METHOD FOR ENHANCING ELASTICITY OF TISSUE

BACKGROUND OF THE INVENTION

The present invention relates to compositions suitable as therapeutics, pharmaceuticals, and/or cosmetics. The composition(s) of the present invention preferably include a compound which is analogous with a portion of mammalian elastin. The present invention also relates to methods of using the compounds and compositions described and contemplated herein to achieve a therapeutic, pharmaceutical, or cosmetic effect. It is preferable that the compound(s) and composition(s) of the present invention be administered at a therapeutically effective concentration and/or as an active ingredient of a pharmaceutical, therapeutic and/or cosmetic preparation.

Elastin is a highly cross-linked complex polypeptide and a major component of elastic fibers present in the tissue of animals. Elastin is known to play a very important role in maintaining tone, structure, and turgor of tissue. Elastin is found in most connective tissue in conjunction with collagen and polysaccharides. Large amounts of elastin can be found in blood vessel walls, particularly in the arch of the aorta near the heart. Elastin is also present in skin, tendons, ligaments, nerves, loose connective tissue, and in the uterus. In normal mammalian skin, specifically human skin, elastic tissue proteins represent a relatively small fraction of the total dermal proteins.

Elastin fibers are capable of stretching to several times their length and then returning to their original size upon release of tension. Accordingly, elastin contributes to the physiological elasticity of tissue. It has been found, for instance, that a loss of elasticity in the skin is associated with decrease in the tone and turgor of the skin. It is speculated that the decrease in skin tone and turgor occurs through degradation of elastin and collagen. The quality of aging skin is affected by a loss in elasticity that occurs gradually. This loss of elasticity is most prominent in the last decades of life. Loss of elasticity is due in part to a change in the quality of connective tissue proteins such as elastin and collagen. The most common signs of aging skin are fine lines and wrinkles, which are partially brought on by exposure to ultra violet radiation, diseases and smoking.

Attempts have been made to use elastin itself as a cosmetic agent, however, the dense cross-linked structure of elastin makes it very difficult to solubilize.

SUMMARY OF THE PRESENT INVENTION

The present invention is directed to compositions that are pharmaceutical, therapeutic, and/or cosmetic in nature. Compositions of the present invention preferably modify or appear to modify the physical characteristics of the tissue to which it is applied.

As described herein, the compound(s) which best accomplish an increase or apparent increase in tissue elasticity and turgor are ones which are analogous to or substantially homologous with portions of elastin. Compounds contemplated within the present invention are those that mimic the action or functionality of amino acid containing peptides or peptide-like compounds of the present invention.

More specifically, the compounds or compositions of the present invention mimic the actions or functionality of a peptide selected from the group consisting of SEQ ID 1, SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 5, SEQ ID 6, SEQ ID 7, SEQ ID 8, SEQ ID 9, SEQ ID 10, SEQ ID 11, SEQ ID 12, SEQ ID 13, SEQ ID 14, SEQ ID 15, SEQ ID 16, SEQ ID 17, SEQ ID 18, SEQ ID 19, SEQ ID 20, SEQ ID 21, SEQ ID 22, SEQ ID 23, SEQ ID 24, SEQ ID 25, SEQ ID 26, SEQ ID 27, SEQ ID 28, SEQ ID 29, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39, SEQ ID 40, SEQ ID 41, SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, SEQ ID 65, SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 70, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74 and SEQ ID 75 and their biological equivalents.

Another embodiment of the present invention is directed to a peptide or peptide-like compound having a formula of R_1 -Valine-Valine-Proline- R_2 , wherein R_1 is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10

amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

Another embodiment of the present invention is directed to a peptide or peptide-like compound having a formula of R₁-Valine-Valine-Proline-Asparagine-R₂, wherein R₁ is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10 amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

Another embodiment of the present invention is directed to a peptide or peptide-like compound having a formula of R₁-Valine-Valine-Proline-Glutamine-R₂, wherein R₁ is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10 amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

Another embodiment of the present invention is directed to a peptide or peptide-like compound having a formula of R₁-Leucine-Glycine-R₂, wherein R₁ is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10 amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

Another embodiment of the present invention is directed to a peptide having a formula of R₁-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-R₂, wherein R₁ is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10 amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

Another embodiment of the present invention is directed to a method of enhancing the functionality, tone, turgor, and/or elasticity of the tissue to which it is administered by administering effective amounts of a peptide to the tissue. When treating skin with the compositions of the present invention, the appearance of the skin is enhanced. It is believed that this enhancement is a consequence of improving the elasticity and overall

appearance of the skin. It is preferable that the administration step be comprised of a number of separate steps which are repeated over a predetermined time (e.g., twice daily for one week). It is preferable that the predetermined time exceeds one week of daily administration of the compound, more preferably two weeks, and most preferably at least a month of daily topical application (with twice daily of the peptide administration over the month being even more preferable).

The compounds and agents described herein are preferably administered at an effective concentration within a therapeutic, pharmaceutical or cosmetic composition. The therapeutically effective concentration of the compound(s) (i.e. the peptide or peptide-like compounds) is preferably in a range of about .0002% to about 90% by weight, more preferably in a range of about .05% to about 50% by weight, even more preferably in a range of about 0.5% to about 10%, even more preferably in the range of about 1.0% to about 2.0%, and even more preferably about 1.8% by weight.

The composition of the present invention can be formulated as a cosmetic preparation to be applied topically to the skin, such as in an emulsion, lotion, spray, powder, ointment, cream, or foam or in other suitable pharmaceutical vehicles or carriers commonly known in the art for other types of administration (e.g., oral or subcutaneous). The delivery system of the present invention is preferably a topical delivery system but also may be a subcutaneous, transcutaneous, oral, nasal, aerosol, or patch. The compositions of the present invention have many other applications. For example, they may also be used to coat surgical devices such as stents and the like.

The composition of the present invention may be suitable to treat a variety of diseases or conditions selected from the group consisting of conditions or diseases of the skin, tendons, sheaths and/or bone, hair, lip, back or spine, brain or nervous system, autoimmune system, lungs, muscle, joints, nails, blood vessels/lymphatics, breast, cartilage, ear, eye, genito-urinary tract, gastrointestinal tract, immunologic systems, ulcerative, blood vessels/heart (e.g., hypertension), and other body systems.

BRIEF DESCRIPTION OF THE DRAWINGS

The features, aspects, and advantages of the present invention will become better understood in light of the following description, appended claims, and accompanying drawings wherein:

Fig. 1 is a bar graph illustrating increased elastin production as a result of application of select compounds of the present invention to mammalian skin.

Fig. 2 is a micrograph illustrating the microvascular response of the skin tissue with peptides of the present invention: Fig. 2a is a representative sample from unshaven control tissue; Fig. 2b is a representative sample from shaven control tissue; Fig. 2c is a representative sample of tissue which received DHEA topical treatment; Fig. 2d is a representative sample of tissue treated topically with peptides of the present invention.

Fig. 3 is a bar graph illustrating tritiated Thymidine incorporation with selected peptide or peptide-like compounds.

Fig. 4 illustrates an elastin rtPCR assay;

Fig. 5 illustrates quantification of newly synthesized tropoelastin visualized with an anti-tropoelastin antibody and FITC-labeled conjugate. (A) Basal level of tropoelastin (green) produced by non-treated adult human dermal fibroblasts. (B) Increased level of tropoelastin (green) after incubation with HEP for 24 hours. (C) Tropoelastin levels increase with time above basal level;

Fig. 6 shows human skin treated with the composition of the present invention (i.e. 2% HEP). Top panels are as indicated, before and after, H&E stained tissue sections for nuclei. The lower panels are before and after, Verhoff's stain for elastin; and

Fig. 7 is a bar graph illustrating the measurement of elasticity of the skin by a Cutometer with various formulations in accordance with the present invention.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

So that the invention described herein may be more fully understood, the following detailed description is set forth. The description is in no way meant to limit the breadth of the claims, but rather to specifically point out novel aspects of the present invention.

The present invention relates to compositions useful in increasing functionality, elasticity, tone, turgor, and/or appearance of tissue. The present invention is also directed to administering therapeutically effective concentrations of the compositions.

Definitions Useful in Understanding the Invention: As used herein, the term "subject" or "patient" means any mammal in which elastin is utilized for proper tissue function or appearance. The methods herein for use contemplate prophylactic, cosmetic, and curative use.

As used herein, the term "about" means plus or minus 10% of the numerical value of the number with which it is being used. Therefore, about 50% means in the range of 45%-55%. As used herein, the term "Dalton" (or "Da") refers to the unit of mass which is equivalent to the mass of a hydrogen atom (1.66×10^{-24} gram).

Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function. The term "tissue", as usually used herein, refers to tissue which includes elastin as part of its structure and/or function. For example, connective tissue which is made up of, among other things, collagen fibrils and elastin fibrils satisfies the definition of "tissue". Additionally, since elastin appears to be inherently involved in the visco-elasticity of blood vessels, veins, and arteries, these would be encompassed in the definition of "tissue". The term "skin" is encompassed by the term "tissue" but specifically means the outer integument or covering of the body, including the dermis and the epidermis which rests upon subcutaneous tissue.

The term "elastin peptide fragment" in either singular or plural form herein refers to the fact that the peptide or amino acid sequence being discussed corresponds to, is the biological equivalent of, is analogous with, or is substantially homologous with, a portion of elastin. The term "elastin peptide fragment" is not meant to convey any meaning regarding the source or starting material or method of arriving at the elastin peptide fragment.

As used herein, the term "providing" when used in conjunction with a therapeutic, pharmaceutical, or cosmetic, means to administer an agent directly into or onto a target tissue or to administer a therapeutic to a patient whereby the therapeutic positively impacts the tissue to which it is targeted (either in a prophylactic, curative or cosmetic manner). Thus, as used herein, the term "providing", when used in conjunction with an elastin peptide fragment and/or a skin enhancing agent, can include, but is not limited to, providing the compound(s) into or onto the target tissue; providing an elastin peptide fragment systemically to a patient (e.g., by providing intravenous injection whereby the therapeutic agent reaches the target tissue); and providing the therapeutic agent in the form of the encoding sequence thereof to the target tissue (e.g., by so-called gene-therapy techniques) whereby the elastin peptide fragment and/or skin enhancing agent is expressed within the target tissue. Details on techniques for formulation and administration of pharmaceuticals may be found in the latest edition of *Remington's Pharmaceutical Sciences* (Mack Publishing Co, Easton Pa.). Although local topical delivery is desirable, there are other acceptable means of delivery, for example: oral, parenteral, aerosol, intramuscular, subcutaneous, transcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration.

As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve a condition or disease of a patient. A particular condition treated in the present invention is deficient elastin in a particular tissue, that is, a need in the tissue for more elastin. As it applies to skin, "therapy" is often measured by turgor, tone, appearance, degree of wrinkles, and youthfulness. As the term applies to blood vessels it may be measured by the degree of elasticity or proper vasomotor response (vasodilatation/vasoconstriction) of the vessel. Accordingly, therapeutic treatment of blood vessels may have implications in diseases associated with visco-elasticity, including

hypertension, arteriosclerosis, angina, angiogenesis, myocardial infarction, coronary thrombosis, restenosis post angioplasty, and chronic obstructive pulmonary disease.

The compounds and compositions of the present invention may also be useful as an agent for modifying tissue, especially skin. The term "modify" is used to convey that the present invention changes either the appearance, form, characteristics and/or the physical attributes of the tissue to which it is being provided, applied or administered. The change in form can be reflected in any of the following, alone or in combination: enhanced appearance of the skin; increased softness of the skin; increased turgor of the skin; increased texture of the skin; increased elasticity of the skin; decreased wrinkle formation and increased endogenous elastin production in the skin.

Finally, the term "cosmetic," as used herein, refers to a beautifying substance or preparation which preserves, restores, bestows, simulates, or enhances the appearance of bodily beauty, specifically as it relates to the appearance of tissue or skin.

Initial Methods, Materials, and Formulations: Elastin itself can be used as starting material in the digestion or cleavage methods described herein to arrive at the peptide portion of the composition. This elastin can be derived from a number of sources known in the art. The sequences of the present invention can either be isolated from the digestion pool (and chemically modified if desired) or the peptides may be synthesized with a peptide synthesizer. A particularly useful source of elastin is *ligamentum nuchae*. *Ligamentum nuchae* contains large amounts of elastin (approximately 70% of the dry weight of this ligament is elastin), especially in proportion to the amount of collagen. Due to the relatively high elastin content and relatively low collagen content, *ligamentum nuchae* is an ideal starting material to use in deriving the elastin peptide fragments of the present invention. *Ligamentum nuchae* may be cleaned first using a procedure similar to that disclosed in U.S. Patent No. 5,028,695, the cleaning portion of which is incorporated herein by reference thereto. Although a preferred source of starting elastin is *ligamentum nuchae*, other ligaments, tendons, connective tissue, tissue, and synthetic sources may also be used. For example, the arteries and lungs, and other animal tissue, especially those which have significant amounts of elastin, can be used (e.g., rat, sheep, and porcine aorta can be used as a

source of elastin as described in L.B. Sandberg, *Connective Tissue Research*, 1990, Vol. 25, pp. 139-148, incorporated herein in its entirety by reference thereto). Also, elastin from different sources, or elastin and collagen from the same or different sources could be mixed together to produce a particular advantageous mix suitable for digestion or hydrolytic cleavage.

The ligament extraction process is comprised of taking dissected *ligamentum nuchae* ligaments and removing as much fat and excess connective tissue as possible. These "clean" ligaments are then chopped into about one centimeter square (1 cm²) pieces and washed with doubly distilled water ("DDW"). The clean ligaments are then placed on a metal mortar and pre-chilled to -20°F. Liquid nitrogen is added to freeze the tissue. The ligaments are then minced or pulverized with the appropriate tool and re-suspended in 1% aqueous NaCl at a ratio of about 100 grams of tissue to about three liters of 1% aqueous NaCl and homogenized in a Waring blender at high speed for 30-60 seconds. The homogenized ligament is transferred to a four-liter beaker and stirred overnight at 4°C on a magnetic stirrer, after which it is centrifuged at 32,500 x G and a pellet is formed. The supernatant is checked for protein content using the Biuret method for protein determination. The Biuret reaction is done by mixing 2 milliliters of extract with 3 milliliters of reagent and immediately reading the protein concentration of the supernatant either by simple visual inspection or at 540 nanometers on a spectrophotometer. The supernatant is then discarded. The pellet (referred to hereinafter as the elastin pellet) is resuspended in 1% aqueous NaCl and homogenized. The process of homogenizing in a Waring blender, stirring overnight and centrifuging are repeated three to four times until the supernatant is Biuret negative. After centrifugation, the elastin pellet is resuspended in DDW and autoclaved at 30 psi for six hours. The resuspended elastin pellet is centrifuged again and the supernatant is tested for protein content via the Biuret method. The elastin is washed with boiling DDW and then with DDW at room temperature. The washes are tested for protein content via the Biuret method. If the washes are Biuret negative, the elastin pellet is dried with chloroform/methanol solution at a ratio of 2 parts chloroform to 1 part methanol. If the Biuret test is positive, the six hour autoclave procedure with wash step is repeated until the Biuret test is negative. Finally, the elastin residue is washed with five volumes of pure methanol and air-dried at room temperature. The elastin residue is transferred to a desiccator and dried over P₂O₅ for 24 hours or until the

weight of the elastin residue is stable. The elastin residue is then milled in a Willey mill through a 40-mesh screen followed by a 60-mesh screen.

For the thermolysin digestion, three times re-crystallized thermolysin product from CalBiochem (10394 Pacific Center Court, San Diego, CA 92121) was used. The thermolysin preparation contains sufficient calcium to ensure maximal activity of the enzyme. The thermolysin digestion is done as follows: a waterbath is brought to a 55 °C temperature with a rotary shaker and five grams of the finely milled largely insoluble elastin residue is hydrated with one liter of DDW for fifteen minutes at room temperature. After hydration, the one liter of DDW which contains the five grams of elastin is placed in the 55 C bath and the pH of the elastin/water mixture is brought to between 7 and 8 with 10% methylamine. Fifty milligrams of thermolysin (*Bacillus thermoproteolyticus*) is added directly to the elastin/water mixture. The thermolysin contains about 60% protein (60.2%), about 13% (13.2%) sodium acetate, and about 25% (25.3%) calcium acetate, with a specific activity of about 8,720 I.U./mg dry weight. The pH of the elastin water mixture is monitored with a pH meter or pH stat and adjusted with 10% methylamine to keep the pH between 6.8 and 7.5. The digestion is allowed to continue for 75 minutes. Concentrated hydrochloric acid is then added to adjust the pH to 3.0 to terminate the digestion.

After digestion is terminated, the digested product is preferably filtered through a PM 10 Diaflow 10,000 molecular weight cut-off ultra-filtration membrane to filter out any protein or peptides exceeding about 10,000 Da molecular weight. The resulting supernatant is a derived composition comprised of peptides having a molecular weight of less than about 10,000 Da. This step is useful in removing any unwanted higher molecular weight material from the compositions of the present invention. This may be particularly useful in removing any potentially harmful higher molecular weight agents, such as prions or other high molecular weight pathogens.

The elastin peptide fragment/water mixture (inclusive of SEQ IDs 1-41 in Table I shown below) which is obtained upon digestion with thermolysin described above is flash evaporated to dryness, redissolved in a small volume of DDW, and if desired, diluted sufficiently with DDW for lyophilization to dryness. In the alternative, rather than

redissolving the elastin peptide(s), the filtered product is freeze dried twice, resulting in a powder which contains 30 weight % chemically-bound water and very little salt (NaCl). Table I is a list of peptide sequences which in combination with other skin enhancing agents exhibit desirable characteristics. It should be noted that SEQ IDs 23 and 32 appear to be a common sequence because Proline hydroxylation is a post-translational event.

A typical cream incorporating SEQ IDs 1-41 of the present invention is described as follows in order of relative concentration of each of the ingredients: Purified Water, Hydrolyzed elastin peptides ("HEP") (i.e., SEQ IDs 1-41), Polyglycerylmethacrylate (and) propylene glycol, Petrolatum, Dicaprylylether, Peg- 5, Glycerylstearate, Glycerine, Dimethicone (and) dimethiconal, Cetyl alcohol, Sweet almond oil, Acrylates/C10-30, Tocotheryl acetate, Phenoxyethanol, Benzyl alcohol, Disodium EDTA, Sodium hydroxide, Lactic acid, Sodium chloride. The HEP is often stated in weight percentage (e.g., 2% HEP is 2 wt/wt% HEP)

TABLE I

| SEQ # | PEPTIDE | MOL WT | NAME (N- to C-terminal) |
|-------|---------|-----------|---------------------------------------|
| 1. | AVG | 245 | Alanine-Valine-Glycine |
| 2. | VGAG | 302 | Valine-Glycine-Alanine-Glycine |
| 3. | IGG | 302 | Isoleucine-Glycine-Glycine |
| 4. | LG | 188 | Leucine-Glycine |
| 5. | IGAG | 316 | Isoleucine-Glycine-Alanine-Glycine |
| 6. | LGG | 245 | Leucine-Glycine-Glycine |
| 7. | VAPG | 342 | Valine-Alanine-Proline-Glycine |
| 8. | LGPG | 342 | Leucine-Glycine-Proline-Glycine |
| 9. | LGAG | 316 | Leucine-Glycine-Alanine-Glycine |
| 10. | VGPG | 328 | Valine-Glycine-Proline-Glycine |
| 11. | FGPG | 376 | Phenylalanine-Glycine-Proline-Glycine |

| | | | |
|------|---------|-----|---|
| 12. | VGPQ | 399 | Valine-Glycine-Proline-Glutamine |
| 13. | LGA | 259 | Leucine-Glycine-Alanine |
| 14. | VGPA | 342 | Valine-Glycine-Proline-Alanine |
| 15. | VVPG | 370 | Valine-Valine-Proline-Glycine |
| 16. | AVPG | 342 | Alanine-Valine-Proline-Glycine |
| 17. | VVPQ | 441 | Valine-Valine-Proline-Glutamine |
| 18. | VAARPG | 569 | Valine-Alanine-Alanine-Arginine-Proline-Glycine |
| 19. | LGAGGAG | 501 | Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine |
| 20. | AIPG | 356 | Alanine-Isoleucine-Proline-Glycine |
| 21. | LGPGG | 399 | Leucine-Glycine-Proline-Glycine-Glycine |
| 22. | AAAQA | 430 | Alanine-Alanine-Alanine-Glutamine-Alanine |
| 23. | VGVBypG | 444 | Valine-Glycine-Valine-Hydroxyproline-Glycine |
| 24. | VYPGG | 491 | Valine-Tyrosine-Proline-Glycine-Glycine |
| 25. | IGGVGG | 458 | Isoleucine-Glycine-Glycine-Valine-Glycine-Glycine |
| 26. | VAPGVG | 498 | Valine-Alanine-Proline-Glycine-Valine-Glycine |
| 27. | LGVGG | 401 | Leucine-Glycine-Valine-Glycine-Glycine |
| 28. | VLPG | 384 | Valine-Leucine-Proline-Glycine |
| 29. | FRAAA | 534 | Phenylalanine-Arginine-Alanine-Alanine-Alanine |
| 30. | VGGVPG | 484 | Valine-Glycine-Glycine-Valine-Proline-Glycine |
| 31. | FGPGG | 433 | Phenylalanine-Glycine-Proline-Glycine-Glycine |
| 32.* | VGVPG | 427 | Valine-Glycine-Valine-Proline-Glycine |
| 33. | VLPGAG | 512 | Valine-Leucine-Proline-Glycine-Alanine-Glycine |

| | | | |
|-----|---------|-----|--|
| 34. | VGLHypG | 458 | Valine-Glycine-Leucine-Hydroxyproline-Glycine |
| 35. | LGVGA | 415 | Leucine-Glycine-Valine-Glycine-Alanine |
| 36. | AFPG | 390 | Alanine-Phenylalanine-Proline-Glycine |
| 37. | AFPGA | 461 | Alanine-Phenylalanine-Proline-Glycine-Alanine |
| 38. | VGIPA | 455 | Valine-Glycine-Isoleucine-Proline-Alanine |
| 39. | VGGIPT | 542 | Valine-Glycine-Glycine-Isoleucine-Proline-Threonine |
| 40. | VGVGVP | 583 | Valine-Glycine-Valine-Glycine-Valine-Proline-Glycine |
| 41. | LGPGVG | 498 | Leucine-Glycine-Proline-Glycine-Valine-Glycine |

It would appear that certain groups of the peptides described in Table I (SEQ IDs 1-41, inclusive) have preferred characteristics as they relate to cosmetic, pharmaceutical or therapeutic application to the skin. The elastin peptide mixtures isolated from thermolysin digestion of elastin (i.e., SEQ IDs 1-41, inclusive) were collected as they came off of a HPLC column. Instead of isolating each of the thermolysin peptide fragments individually, 5 fractions or clusters of peptides were collected in the 5-50 minute range and tested for activity utilizing a bromodeoxyuridine Triphosphate (BrdUTP) incorporation assay. The assay measures production of mRNA involved in protein synthesis. Table II measures the green fluorescence intensity as a measure of increased mRNA in RFL-6 cells in response to the pooled elastin fragment.

TABLE II

| <u>Fraction #</u> | <u>Approximate Elution time</u> | <u>Approximate % Change Minus</u> <u>Control</u> |
|-------------------|---|---|
| 1 | 5.3 min. – 11.8 min | 1% |
| 2 | 11.8 min – 23.0 min | 4% |
| 3 | 23.0 min – 44.1 min | 41% |
| 4 | 44.1 min – 45.8 min | 10% |
| 5 | 45.8 min – 50.0 min | 2% |
| 6 | Unfractionalized mixture (SEQ IDs 1-41) | 52% |

Each of the fractions show an increase in mRNA in RFL-6 cells over the control group. From the test, however, it appears that Fraction #3 alone and/or in combination with other fractions (e.g., as seen with Fraction #6) has a preferred composition when increasing elasticity, turgor, and/or appearance of tissue, specifically skin. Fraction 3 includes SEQ IDs 14-31. It should be noted that in light of the ease in obtaining the unfractionalized mixture (as described above) it may be more preferable to use the unfractionalized mixture than to isolate the most active ingredient.

Fraction or Cluster 3 was sub-fractionated into 10 fractions corresponding to the ten major peaks identified on the HPLC (at 215 nm). Table III below illustrates the green fluorescence intensity as a measure of increased mRNA in RFL-6 cells in response to sub-fractionated portions of Fraction No. 3.

TABLE III

| <u>Fraction #</u> | <u>Expected Seq. No. in Fraction</u> | <u>Peptide Sequence</u> | <u>% Change Green Fluorescence Intensity</u> |
|-----------------------|--|---------------------------------------|--|
| 1 | SEQ ID 14 | VGPA | 39 |
| 2 | SEQ IDs 15, 16 | VVPG, AVPG | 40 |
| 3 | SEQ ID 17 | VVPQ | 85 |
| 4 | SEQ IDs 18, 19 | VAARPG, LGAGGAG | 44 |
| 5 | SEQ IDs 20, 21 | AIPG, LGPGG | 42 |
| 6 | SEQ ID 22 | AAAQA | 20 |
| 7 | SEQ ID 23 | VG VHypG | 57 |
| 8 | SEQ ID 24 | VYPGG | 38 |
| 9 | SEQ IDs 25, 26, 27, 28, 29 | IGGVGG, VAPGVG, LGVGG, VLPG, FRAAA | 10 |
| 10 | SEQ IDs. 30, 31 | VGGVPG, FGPGG | 23 |
| Blank (Background) | — | — | 30 |

As can be seen from Table III, it appears SEQ ID 17 (VVPQ) has the greatest activity, followed by SEQ ID 23 (VG VHypG) and then SEQ IDs 18 (VAARPG) and 19 (LGAGGAG). It would appear that SEQ IDs. 22 and 25-31 may actually have an adverse impact the overall therapeutic or cosmetic value of Fraction 3, at least to the extent the therapeutic value is attributed to elastin production. However, applicant does not wish to be bound by theory or speculation in that any fraction or combination of fractions, while lowering the green fluorescence intensity of the fractionated sample, may in fact add a desirable characteristic to the intended use of the overall mixture when combined with another peptide (e.g., any of SEQ IDs 1-41, respectively). Other types of testing may in fact demonstrate the suitability of other peptides disclosed herein for pharmaceutical and/or cosmetic purposes.

The present invention can be formulated in a number of carrier vehicles, for example, in a spray; an aerosol; a water and an oil-type emulsion; an oil and water-type emulsion; a face cream or body cream; a sun lotion or after-sun lotion; or other topical administration vehicle. U.S. Patent No. 4,327,078, which was referenced earlier, is illustrative of the different types of topical administrations which may be employed to administer a soluble elastin-based derivative, and is incorporated herein by reference for this purpose. The method of administering peptides and formulations of the present invention employs any of a number of known administrative routes such as oral, IV, subcutaneous, transcutaneous, and topical administration. A preferred method of the present invention employs a pharmaceutical or cosmetic composition which enhances the physical appearance of and/or the elasticity of tissue. Compositions of the present invention may be in the form of a peptide or peptides in combination with at least one other agent, such as stabilizing compound, which may be administered in any sterile, bio-compatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones. Pharmaceutically-acceptable carriers may also be comprised of excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of *Remington's Pharmaceutical Sciences*. The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

With the aforementioned wide-spread applicability in mind, a number of peptide or peptide-like compounds were isolated and/or synthesized and analyzed as potential therapeutic, pharmaceutical, or cosmetic agents.

As can be seen from Fig. 1, the topical treatment with a composition which included peptide fragments (i.e., SEQ IDs 1-41) at a concentration of about 1.3% (wt/wt) of

the formulation when applied to the skin of a Sprague-Dawley male rat over a one month period illustrates a doubling of the elastin content of the skin, as compared to both control samples and similar applications and concentration of DHEA. In Figure 1, S CONTR represents the Shaven Control and US CONTR represents the Unshaven Control. Fig. 1 illustrates that the compounds of the present invention have the advantageous qualities of enhancing the softness or elasticity of the skin. The peptides and formulations of the present invention also appear to improve the texture of skin, specifically the physical appearance of the skin.

The Sprague-Dawley rats used to generate Fig. 1 were treated topically with a 1.3% concentration (wt/wt) of the preparation of the hydrophilic elastin peptide for a period of 30 days. Testing illustrated that the endogenous elastin (measured by microgram (μg) Elastin per milligram (mg) Skin Fat Free Dry weight) of each of the rats to which the composition was applied doubled over that of a control sample and a sample which was treated with a 5% concentration of DHEA over a similar time period. Three animals each were used to generate the data for S CONTR, US CONTR, and DHEA. Eleven animals were used for HEP. Three skin samples from the treated areas of each animal were taken for study, and the three results from each animal were averaged. The means of these results are as follows: S CONTR (1.408); US CONTR (2.291); DHEA (1.753); HEP (3.175). The elastin content of the skin was determined by a precise assay for rat elastin developed by Sandberg, et al. (Connective Tissue Research. 25: 139-48, 1990) the assay portion of which is hereby incorporated herein by reference thereto. An alpha level less than 0.001 for the data of Fig. 1 as determined by analysis of variance is significant because there is less than one chance in a thousand that the findings occur by chance. The data of Fig. 1 further supports the use of the cosmetic or pharmaceutical preparation over an extended period preferably in the range of one week to one month, more preferably in the range of seven days to about fourteen days and most preferably about fourteen days of daily administration at about 1.5% concentration (wt/wt) of elastin peptide or peptides.

Fig. 2 is a micrograph illustrating an increased appearance and beneficial implication of the present invention. From Fig. 2, skin treated with an elastin peptide fragment appears to be healthier than untreated skin. This is evidenced under a microscope

by an increase in vascular response. In Fig. 2, fixed tissue sections of rat skin were labeled with fluorescein conjugated antifibronectin antibodies. Fig. 2a is a representative sample from the unshaven control tissue; Fig. 2b is a representative sample from the shaven control sample; and Fig. 2c is a representative sample of the tissue which received DHEA topical treatment. Finally, Fig. 2d received treatment with the present invention in a topical form in accordance with the samples discussed above with regard to Fig. 1. The dermal layer in the control panels (Figs. 2a and 2b) is relatively uniform and thin compared to the thickness of both Figs. 2c and 2d. For convenience, in each of panels Figs. 2a - 2d, the dermal layer is bracketed. Surprisingly, panel Fig. 2d illustrates an increased concentration of capillary venules in the subdermal region. The capillary venules are shown in this figure as brightly stained oval bodies that lie beneath the dermal layer. The increase in the concentration of endothelial cells in the subdermal region indicates an increase in capillary density and therefore illustrates the potential for the peptides and formulations of the present invention to be used for the formation of blood vessels or capillary venules. Thus, compositions of the present invention may be useful in neovascularization or angiogenesis.

Modification of Active Peptides: The bar graph of Fig. 3 illustrates the effect of modifying sequences in a variety of ways. The results of modifying SEQ ID 17 (what appears to be the most active peptide for many purposes) provide important information on the impact of such modifications. For instance, modification of SEQ ID NO 17 which result in SEQ ID NOs 42 and 43 appear to adversely impact the suitability for these purposes. SEQ ID 4 (LG) resulted in about an 8% CPM above the control; SEQ ID 17 (VVPQ) resulted in about a 28% CPM above the control; SEQ ID 19 (LGAGGAG) resulted in about an 18% CPM above the control; SEQ ID 42 (VVPQ-NH₂) resulted in about a 1% CPM above the control; SEQ ID 43 (Acetyl-VVPQ) resulted in about a 1% CPM above the control; SEQ ID 48 (GAVVPQ--NH₂) resulted in about a 25% CPM above the control; and SEQ ID 44 (Acetyl-GAVVPQ--NH₂) resulted in about a 5% CPM above the controls. From Fig. 3 and the genetic expression data presented herein, it appears that the synthetic peptide SEQ ID 17 appears to have the same or nearly the same activity as SEQ ID 17 isolated from the HPLC fractionalization. Accordingly, focus should be placed upon this peptide. It would also appear that a GA residue attached to the N-terminus of the SEQ ID 42 (resulting in SEQ ID

48) has a similar activity to the activity of SEQ ID 17. The ubiquity of the GA residue in an elastin's peptide sequence suggests that such a modification of other peptide fragments may augment their activity and/or otherwise may be desirable. Having an amide at the carboxyl terminus or an acetyl at the N-terminus may also beneficially affect activity and/or solubility of the subject peptide.

The information derived from Table III and Fig. 3 was utilized to systematically synthesize peptides which would appear to be particularly suitable as pharmaceutical, cosmetic, and/or therapeutic compositions. A general method for synthesizing peptides is described in U.S. Patent No. 4,816,513, incorporated herein by reference thereto in its entirety, which describes a process for automatically constructing a polypeptide. Additionally, U.S. Patent No. 4,668,476, incorporated herein by reference thereto in its entirety, also describes an apparatus for automatically constructing a polypeptide and a transfer system to transfer activated species from the activator system to the reaction vessel and to transfer amino acids, reagents, gases and solvents from one part of the apparatus to another. Generally, this synthesis process is conducted using Fmoc chemistry on automated solid phase synthesizers, (or in some cases by Boc chemistry). In most cases, the synthesized peptides would be purified by HPLC using reversed phase C4 and C18 columns. Alternate purification methods include ion exchange and gel filtration chromatography.

Our studies indicate that sequences which include the critical residue VVP have enhanced activity. SEQ ID 17 (VVPQ), for example, showed particularly good activity. Derivatives of SEQ ID 17 were synthesized. Table IV illustrates the three types of derivatives of SEQ ID 17 which were synthesized and determined to be suitable as pharmaceutical, therapeutic, and or cosmetic compositions in accordance with the present invention. SEQ IDs 45-48 illustrate various modifications of VVPQ at either the amino terminus or carboxy terminus of the peptide. SEQ IDs 49-51 have been modified to include a cysteine residue at both the carboxy and amino terminus of the peptides. The cysteine residues provide a sulfhydryl group at each end of the chain which permits convenient formation of cyclic disulfide. Finally, SEQ IDs 52-54 are very similar to SEQ IDs 49-51, but they have copper as a chelating agent to form a cyclic structure.

TABLE IV (VVPO derived peptides)

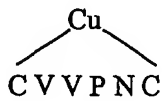
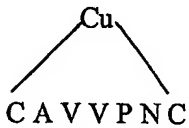
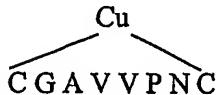
| SEQ # | PEPTIDE | MOL WT | NAME (N- to C-terminal) |
|-------|---|-----------|---|
| 42 | VVPQNH ₂ | 448 | Alanine-Valine-Proline-Glutamine-Amide |
| 43 | (CH ₃ CO)VVPQ | 475 | Acetyl-Valine-Valine-Proline-Glutamine |
| 44 | (CH ₃ CO) GAVVPQNH ₂ | 610 | Acetyl-Glycine-Alanine-Valine-Valine-Proline- Glutamine-Amide |
| 45 | AVVPQ | 512 | Alanine-Valine-Valine-Proline-Glutamine |
| 46 | GAVVPQ | 569 | Glycine-Alanine-Valine-Valine-Proline-Glutamine |
| 47 | AVVPQNH ₂ | 519 | Alanine-Valine-Valine-Proline-Glutamine-amide |
| 48 | GAVVPQNH ₂ | 576 | Glycine-Alanine-Valine-Valine-Proline-Glutamine- amide |
| 49 | CVVPQC | 647 | Cysteine-Valine-Valine-Proline-Glutamine-Cysteine |
| 50 | CAVVPQC | 718 | Cysteine-Alanine-Valine-Valine-Proline-Glutamine- Cysteine |
| 51 | CGAVVPQC | 775 | Cysteine-Glycine-Alanine-Valine-Valine-Proline- Glutamine-Cysteine |
| 52 | $\begin{array}{c} \text{Cu} \\ \diagup \quad \diagdown \\ \text{C V V P Q C} \end{array}$ | 64 | Copper |
| | | 647 | Cysteine-Valine-Valine-Proline-Glutamine-Cysteine |
| 53 | $\begin{array}{c} \text{Cu} \\ \diagup \quad \diagdown \\ \text{C A V V P Q C} \end{array}$ | 64 | Copper |
| | | 718 | Cysteine-Alanine-Valine-Valine-Proline-Glutamine- Cysteine |
| 54 | $\begin{array}{c} \text{Cu} \\ \diagup \quad \diagdown \\ \text{C G A V V P Q C} \end{array}$ | 64 | Copper |
| | | 775 | Cysteine-Glycine-Alanine-Valine-Valine-Proline-Glutamine- Cysteine |

Based on the information gleaned through testing of derivatives and genetic expression data, "VVP" appears to be an important residue.. SEQ ID 55 was synthesized to

replace the glutamine of SEQ ID 17 with an asparagine (Asp - "N") residue, the glutamine residue and asparagine residue having similar charge properties.

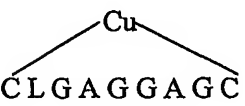
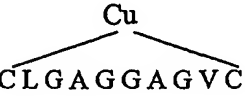
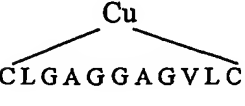
Modifications were made to SEQ ID 52 that were very similar to those made to SEQ ID 17. These modified or synthetic peptides are illustrated in Table V.

TABLE V (VVPN derived peptides)

| SEQ # | PEPTIDE | MOL WT | NAME (N- to C-terminal) |
|-------|---|-----------|--|
| 55 | VVPN | 427 | Valine-Valine-Proline-Asparagine |
| 56 | AVVPN | 498 | Alanine-Valine-Valine-Proline-Asparagine |
| 57 | GAVVPN | 555 | Glycine-Alanine-Valine-Valine-Proline-Asparagine |
| 58 | AVVPNNH ₂ | 505 | Alanine-Valine-Valine-Proline-Asparagine-Amide |
| 59 | GAVVPNNH ₂ | 562 | Glycine-Alanine-Valine-Valine-Proline-Asparagine-Amide |
| 60 | CVVPNC | 633 | Cysteine-Valine-Valine-Proline-Asparagine-Cysteine |
| 61 | CAVVPNC | 704 | Cysteine-Alanine-Valine-Valine-Proline-Asparagine-Cysteine |
| 62 | CGAVVPNC | 761 | Cysteine-Glycine-Alanine-Valine-Valine-Proline-Asparagine-Cysteine |
| 63 |  | 64 633 | Copper Cysteine-Valine-Valine-Proline-Asparagine-Cysteine |
| 64 |  | 64 704 | Copper Cysteine-Alanine-Valine-Valine-Proline-Asparagine-Cysteine |
| 65 |  | 64 761 | Copper Cysteine-Glycine-Alanine-Valine-Valine-Proline-Asparagine-Cysteine |

Since SEQ ID 19 (Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine) also indicated enhanced activity, it was used as a base model for the synthesis of the peptides shown in Table VI below.

TABLE VI (LGAGGAG derived peptides)

| SEQ # | PEPTIDE | MOL WT | NAME (N- to C-terminal) |
|-------|---|-----------|--|
| 66 | LGAGGAGV | 600 | Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine |
| 67 | LGAGGAGVL | 713 | Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine |
| 68 | LGAGGAGVNH ₂ | 607 | Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Amide |
| 69 | LGAGGAGVLNH ₂ | 720 | Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine-Amide |
| 70 | CLGAGGAGC | 707 | Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Cysteine |
| 71 | CLGAGGAGVC | 806 | Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Cysteine |
| 72 | CLGAGGAGVLC | 919 | Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine-Cysteine |
| 73 |  | 64 | Copper |
| | | 707 | Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Cysteine |
| 74 |  | 64 | Copper |
| | | 806 | Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Cysteine |
| 75 |  | 64 | Copper |
| | | 919 | Cysteine-Leucine-Glycine-Alanine-Glycine-Glycine-Alanine-Glycine-Valine-Leucine-Cysteine |

In Vitro and In Vivo Analysis of Select Active Peptides:

Example 1: The peptides VVPQ (SEQ ID 17) and LGAGGAG (SEQ ID 19) were further analyzed using human skin fibroblasts. These peptides have striking and distinct influences on gene expression patterns of these cultured skin fibroblasts. In one study, human skin fibroblasts were grown to confluency in T75 cell-culture flasks (2 passages) using Dulbecco's modified essential media (DMEM) complemented with 10% fetal calf serum (FCS), penicillin, streptomycin and neomycin. After removing the media, the cells were washed with 10% phosphate buffered saline (PBS) and media supplemented with either 10 µg/ml VVPQ or 10 µg/ml LGAGGAG, respectively. The control culture media contained no added peptides. The fibroblasts were treated in this manner for 4 hours after seeding. The media was subsequently removed and total RNA was extracted using RNA STAT-60 (Tel-Test, Friendswood, TX).

Fifty micrograms of total RNA from each sample (either VVPQ, LGAGGAG, or control) was treated with DNase I at 37°C for 1 hour in order to remove possible genomic DNA contamination. This treated total RNA was then size-separated by electrophoresis through a 1.2% denaturing gel to confirm that no significant degradation of RNA had taken place. Next, 5 µg of treated total RNA from each cell sample was reverse transcribed to single-stranded cDNA using [³²P] dATP and reverse transcriptase, according to the manufacturer's protocol for the Clontech Atlas array (Palo Alto, CA). Column chromatography of the labeled cDNA probes as well as hybridization to the array membranes was also performed according to the manufacturer's protocol. A Molecular Dynamics PhosphorImager SI (Sunnyvale, CA) was used to expose and screen the membranes following hybridization and post-hybridization washing.

The array experiments were performed in two sets: 1. untreated fibroblasts and VVPQ - treated fibroblasts, 2. untreated fibroblasts and LGAGGAG - treated fibroblasts. Each set of data was quantitated, compared and analyzed using either Clontech's AtlasImage (Palo Alto, CA) or Molecular Dynamic's ImageQuant (Sunnyvale, CA) software. A Clontech atlas array (Palo Alto, CA) of 588 cDNAs was utilized to analyze the changes in steady state mRNA levels within all 6 functional cDNA groups using fibroblasts treated and untreated with the elastin peptides VVPQ and LGAGGAG. Autoradiograms of duplicate

arrays probed with ^{32}P -labeled cDNA obtained from total RNA isolated from VVPQ-treated and untreated skin fibroblasts were analyzed using ImageQuant (Sunnyvale, CA) software.

In the DNA array analysis, the autoradiographic signal obtained from each duplicate cDNA set on each filter is compared to the control cDNAs on the same filter. This intensity ratio is then compared for the same duplicate cDNAs between filters. Increased recovery of signal using a cDNA probe from RNA isolated from treated fibroblasts is interpreted as increased mRNA levels. Decreased mRNA levels are observed as a decrease in signal. There were instances where there was no significant hybridization of labeled cDNA to membrane bound duplicate cDNA spots. All array signals that indicated up or down regulation of steady state mRNA levels of at least two-fold in response to VVPQ treatment were identified.

It appears that VVPQ treatment results in an alteration in steady state levels of relatively few mRNAs, any many of those upregulated in steady state levels were involved in either apoptosis or DNA repair. These include caspase 10 and RFC4. No particular functional class of mRNAs appear to predominate among the mRNAs which appear to be downregulated in response to VVPQ treatment. The hypothesis is that VVPQ has a significant influence on apoptosis in these human fibroblast cultures, but the mechanism of this influence is only now being elucidated.

The peptide LGAGGAG appears to result in an increased steady state level of many different mRNAs. Of these, many of the encoded proteins again appear to be involved in either apoptosis or DNA repair. Although the number of mRNAs altered by treatment with LGAGGAG was greater in our initial studies than with VVPQ, an influence by both peptides on apoptosis and or DNA repair is similar. The larger and distinctly different influence of LGAGGAG, however, appears to be on mRNAs encoding proteins involved in cell to cell communication. A large percentage of mRNAs experienced increased steady state levels in response to treatment of fibroblasts with LGAGGAG. These include mRNAs encoding proteins such as endothelin 2, oncostatin, TGF- and BMP2A. Interestingly, the levels of only a few mRNAs were noted to be significantly down-regulated in skin fibroblasts following treatment with LGAGGAG.

The influence of these two peptides (VVPQ and LGAGGAG) on the gene expression patterns of cultured skin fibroblasts appear to be different. While still a preliminary study, it appears that the heptapeptide LGAGGAG has a marked influence on the steady state levels of a larger number and a greater variety of mRNAs in treated fibroblasts than the tetrapeptide VVPQ. While both peptides do influence the levels of mRNAs encoding proteins involved in DNA repair and apoptosis, LGAGGAG appears to have particular influence on mRNAs encoding several different cytokines known to influence the expression of genes responsible for the synthesis of elastin and several different collagen types. It is entirely possible therefore, that the mRNA level changes reflected in this array analysis represent at least part of the signaling cascade influenced by LGAGGAG. The potential signaling cascade influences induced by exposure to the heptapeptide may lead to the connective tissue alterations responsible for phenotypic changes in the skin reported herein. In contrast, the tetrapeptide VVPQ appeared to have a relatively quiescent effect on cultured skin fibroblasts. It must be emphasized that these results are limited by the limited cDNA present in the array used to generate this data. With VVPQ, The mRNAs that are altered in levels are almost entirely those encoding proteins involved in apoptosis and DNA repair. While apoptotic changes can have a profound influence on connective tissue remodeling, this pathway may be different from the one illustrated by the treatment with LGAGGAG which appears to effect levels of mRNAs encoding proteins involved in cell to cell communication. The prediction from these initial results therefore, would be that these two elastin peptides have different influences on the biosynthetic profiles of skin fibroblasts. Even more information could be gained about the effects of these and other elastin peptides on fibroblasts by utilizing a larger array of cDNAs. The differences in gene expression detected by these array analyses may be manifested by significant phenotypic differences on the functional integrity of the dermis and will merit further study.

Example 2 – Elastin mRNA levels and TropeoElastin:

When the hydrophilic elastin peptides (HEP) of the present invention were applied topically they appeared to return elasticity and resilience to skin while helping to lessen wrinkles and fine lines. The in vitro and in vivo effects of the peptides of the present invention follow.

MATERIALS, METHODS, & RESULTS

Elastin mRNA levels. To test the ability of HEP to stimulate the expression of the elastin gene, an rtPCR assay was developed to amplify newly synthesized elastin mRNA. Adult human dermal fibroblasts were grown to approximately 80% confluence prior to being incubated with 10 µg/mL HEP for 2, 4, and 6 hours. A control sample having received no HEP was also run in parallel. After the incubation period, dermal fibroblasts were lysed and total RNA was purified from each sample. Total RNA was quantified and subjected to rtPCR using forward and reverse primers complementary to human elastin mRNA. Amplified cDNA products were then subjected to electrophoresis at 150 volts for 30 min. on a 2% TBE agarose gel containing ethidium bromide. The gel was then photographed and bands were quantified using the Kodak 1D Gel Electrophoresis software. Fig. 4 illustrates this elastin rtPCR assay. Lane (1) shows the net intensity of amplified elastin mRNA from non-treated cells. Lane (2) shows the net intensity of amplified elastin mRNA from treated cells after 2 hours incubation with HEP. Lane (3) shows the net intensity of amplified elastin mRNA from treated cells after 4 hours incubation with HEP. Lane (4) shows the net intensity of amplified elastin mRNA from treated cells after 6 hours incubation with HEP. The net intensities of the PCR products indicate that HEP stimulates nearly a two-fold increase in elastin mRNA expression just after 2 hours of incubating adult human dermal fibroblasts with HEP. Results from this assay indicate that HEP causes an upregulation of the elastin gene in adult human dermal fibroblasts within hours. Specifically, HEP induces nearly a two-fold increase in elastin mRNA after only two hours incubation with adult human dermal fibroblasts as compared to control samples. The stimulus, however, appears to be somewhat transient and elastin mRNA levels begin to decrease in dermal fibroblasts after 4 and 6 hours incubation with HEP.

Tropoelastin levels. Newly synthesized tropoelastin protein levels were measured by quantitative immunocytochemistry in HEP-treated adult human dermal fibroblasts using an LSC (laser scanning cytometer) and a fluorescein-labeled anti-tropoelastin antibody. Adult human dermal fibroblasts were grown to approximately 80% confluence on glass coverslips before being incubated with 10g/mL HEP for 0, 2, 4, 8 and 24 hours. After incubation, 100 µL of 1:100 diluted whole rabbit serum containing anti-human tropoelastin antibodies was added to the dermal fibroblasts after having been fixed in 70%

ethanol for 24 hours. After incubating with whole rabbit serum at 4°C for 24 hours, the cells were washed and fluorescein-labeled anti-rabbit secondary antibody (5 µg/mL final concentration) was added for an additional 24 hours at 4°C. Cells were then washed and counter stained with propidium iodide (5 µg/mL final concentration) for 20 min. before being washed and mounted on slides. Fluorescence was then quantified using a laser scanning cytometer. Results show that tropoelastin increases steadily with time above basal level (See Fig. 5C). Figure 5 shows more green-fluorescent tropoelastin in treated adult human dermal fibroblasts (Figure 5B) than in non-treated (Figure 5A). At 24 hours, there is 15-20% more tropoelastin synthesized by HEP-treated adult human dermal fibroblasts as compared to non-treated (Figure 5C)

Cutometer measurements. Cutometer measurements were performed on patients who were using Elastica Skin Cream (2% HEP) twice daily. Facial Cutometer measurements were performed on the right outer canthus of patients using 2% HEP cream twice daily using a model SEM 575 Cutometer (Courage+Khazaka, Köln, Germany). Measurements were taken every 4 weeks for a period of 12 months. Cutometer measurements were performed using mode one consisting of one skin pull through a 2 mm aperture using 400 mbar of constant negative pressure for 5 seconds. Mode one gave data relevant to skin elasticity. Results showed a mean increase in skin elasticity of 31% after just 4 weeks and 35% after 12 weeks treatment with Elastica Skin Cream. Cutometer measurements taken 30 days post-treatment reveal a continued mean elevation in skin elasticity of 23% from basal Cutometer measurements.

Human skin histologies. Tissue sections from 2mm punch biopsies (before and after 6 weeks treatment with 2% HEP) of the right outer canthus were stained with H&E for morphology and Verhoff's stain for elastin. Images were taken on an Olympus BX-60-based microscope. The microscope was equipped with a 100-watt mercury lamp and epi-fluorescent filters with emission wavelengths of 470nm, 535nm and 630nm. The images were viewed through either a Cohu (black and white, range of magnification 16X-800X) or an OLY-750 analog color camera (range of magnification 4X-200X) and captured with an image processing system (Image Pro Plus, Media Cybernetics, Silver Springs MD). Quantitative morphometry of cell nuclei was also performed using the H&E stained sections. Sections of 2 mm punch biopsies from treated human skin show increased dermal thickness and

increased cell density after six weeks of twice daily use of Elastica Skin Cream. Initial quantitative morphometric determinations estimate an enhanced cellularity of approximately 15%. The differences can be seen in H&E and Verhoeff stained tissue sections of before and after treatment punch biopsies shown in Fig. 6.

DISCUSSION: Both rtPCR and immunocytochemistry confirm that HEP stimulates production of tropoelastin in adult human dermal fibroblasts in vitro. Quantitative morphometric data obtained from histologies of patient punch biopsies confirm that the HEP cream stimulates an increase in dermal cellularity. It is quite possible that increased tropoelastin levels and dermal cellularity combine to enhance skin viscoelasticity thereby causing a faster recovery rate in treated versus non-treated stretched skin. Together these results offer a plausible explanation for observed increases in skin elasticity through Cutometer measurements. While not wishing to be bound by theory, it appears that the peptides of the present invention increase skin elasticity when applied topically to the skin. Moreover, this increase in elasticity translates to a lessening in the appearance of fine lines and wrinkles as seen in before and after treatment photographs. Furthermore, there were no substantial side effects such as redness, peeling, irritation or sun sensitivity in conjunction with use of the composition(s) of the present invention.

Applications of the Present Invention: The peptide or peptide-like compounds of the present invention, as well as their corresponding therapeutic compositions, are expected to have a variety of important applications. The following descriptions provide a brief summary of the conditions these peptide(s) are likely to benefit.

Skin conditions: There are many skin conditions and diseases which would benefit from elastin treatment. Beyond the obvious cosmetic applications (i.e., increased tone, turgor, and appearance), enhanced elastin production will produce long-term beneficial results. For example, the inherited disease Scleroderma is characterized by a thickening and stiffening of the skin, and cutaneous ulcers due to the overproduction of collagen (there are a number of diseases which involve overproduction of collagen and which seem to have an adverse effect on elastin production/content and compromise the tissue). This disease can also have systemic effects on organs and blood vessels. The stiffness and difficulty in motion along with the cutaneous ulcers would benefit greatly from incorporation of elastin into the

skin. A similarly positive outcome would be expected with the treatment of lupus and rheumatoid related skin changes which are generally collagen-vascular diseases involving a decrease in elastin. Herpes Simplex and Shingles are also diseases which would appear to benefit from increased elastin activity.

Other skin conditions would appear to benefit from the present invention. Conditions and problems such as acne rosacea, acne vulgaris, aging skin with vascular fragility, burn treatment, scar contractures from burns, radiation burns, pruritis (or chronic itching), psoriasis, urticaria (commonly referred to as hives), xerosis (abnormal dryness of the skin, eyes or mouth), vesicular dermatoses, cracked fingers and feet, drug eruptions (from an allergic reaction), epidermolysis (a skin condition where the epidermis is in a loosened state, often with the formation of blebs and bullae either spontaneously or after trauma), and erythema multiforme would benefit from treatment with the elastin peptide(s) of the present invention.

There are also inheritable skin disorders such as cutis laxa and EDS or Ehlers Danlos Syndrome (a group of connective tissue disorders in which the skin hangs in loose pendulous folds believed to be caused by decreased elastic tissue formation as well as an abnormality in elastin formation or an excess of collagen), elastoderma, progeria, and pseudoxanthoma elasticum (an inherited disorder in which elastic fibers found in many tissues slowly become calcified) which would benefit from an increase in elastin in the affected tissues.

It is believed that the application of the peptides of the present invention alone or in combination with the tissue enhancing agents of the present invention would result in an increase in tissue elastin and may provide effective treatment for serious diseases such as pemphigus.

Tendons, Sheaths and Bone: Tendons, sheaths and bone all are comprised in part of elastin. Chronic, painful conditions affecting some of these tissues include carpal tunnel disease, fasciitis, flat feet, and tendonitis. These conditions and similar ones will be improved with increased levels of elastin in the affected tissue. Bone spurs, fascial tears,

ligament tears and tendon tears will heal faster with supplemental elastin provided by the elastin peptides of the present invention. These tissues may even become stronger as a result of the expected stimulation of elastin production accompanying this treatment. Additionally, cartilage growth abnormalities may be corrected by application of elastin peptides of the present invention.

Treatment with the compound of the present invention will also be useful in veterinary medicine for skin ulcerations in livestock such as horses and cattle. Hoof problems can be very painful and problematic for horses and other hooved animals. Hoof conditions may benefit from increased keratinocyte production seen with certain compositions of the present invention.

Hair: Hair growth, color, and removal can all be improved by treatment with elastin peptides which will make the hair stronger, more shiny, and improve the condition and healing of irritated skin upon removal of unwanted hair. Premature graying of hair may also be due to decreased elastin.

Lips: Chapped lips and chronic dermatitis or inflammation of the lips can be greatly improved upon treatment with elastin peptides of the present invention. Long-term relief would be a potential benefit from the stimulation of endogenous elastin in these tissues.

Back: The breakdown of elastin in the spine can contribute to herniated disks and lead to acute and/or chronic pain. Replacing elastin with peptides of the present invention along with the stimulation of endogenous elastin could result in improved healing of the disk and reduce or eliminate the pain associated with this condition, especially when combined with other treatments, such as steroids.

Brain and nervous system: In nerve compression syndromes, treatment with elastin peptides of the present invention will likely stimulate endogenous elastin production in certain neurological conditions and promote revascularization after stroke and neural tissue transplants. This revascularization could greatly improve the clinical outcome of these treatments.

Autoimmune diseases: Lupus and other rheumatoid related diseases are characterized by localized destruction or degeneration of elastin in tissues throughout the body. These and similar diseases could greatly benefit from treatment with elastin peptides of the present invention which would promote restoration of damaged tissue and even provide long-term benefit from the stimulation of endogenous elastin.

Lungs: Many lung diseases including chronic obstructive pulmonary disease, laryngeal stenosis, pulmonary fibrosis, pulmonary sarcoid and tracheal stenosis are associated with a decrease in elastin, an important component in maintaining the elasticity and proper functioning of the lung. Often, these lung conditions are due to a decrease in particular proteases which normally balance the activity of elastin-degrading proteases, referred to generally as elastases. An example of this type of deficiency is alpha 1 protease inhibitor deficiency. A decrease in elastin due to this type of deficiency causes a breakdown of the lung matrix which is vital for proper lung function. Other factors, such as smoking can also lead to breakdown of the elastin component of the lung matrix.

Muscle: Muscles are often covered with a thin layer of connective tissue which is comprised of elastin and other components such as collagen. Thus, applying peptides of the present invention to muscle tissue would increase muscle tone and the healing of muscle tears and generally strengthen muscles by increasing their elastin content.

Joints: Similarly, joints are comprised of connective tissue, including elastin. In many cases individuals suffer from joint pain and joint abnormalities as a result of inflammatory disease or from wear and tear which all generally result in decreased amounts of elastin present in the connective tissue of joints. Thus, many joint diseases or problems such as athletic joint injuries, torn cartilage and/or ligaments, osteoarthritis, joint pain, rheumatoid arthritis, and stiff joints could benefit from treatment with elastin peptides of the present invention. These elastin peptides will have the capability to stimulate endogenous elastin in these tissues and may provide substantial and long-term rebuilding and maintenance of the elastin in these tissues.

Nails: Elastin is useful in treating and preventing nail brittleness, split nails, and to enhance the hardness of nails in general. Nails are comprised of flattened epidermal cells and have a high concentration of elastin in the nail bed. Thus, increasing the elastin content of these cells will result in a stronger and more flexible nail.

Blood vessels/ lymphatics: Elastin is an important constituent of vessels. Therefore, application of the peptides to the affected tissues in vascular diseases would appear to result in beneficial therapy. The vascular diseases contemplated include those which involve abnormalities of arteries or veins including atherosclerotic occlusive disease, chronic venous insufficiency, diabetic vasculitis (inflammation of a vessel caused by diabetes), fibrotic mediastinitis associated with superior vena cava syndrome (an exuberant inflammatory sclerogenic process of infectious, rheumatic, hemorrhagic, or undetermined origin, often accompanied by obstruction of mediastinal structure, especially the vena cava), varicose veins, temporal arteritis, stasis dermatitis, and lymphedema (including elephantiasis, which is a chronic unilateral or bilateral edema of the extremities due to accumulation of interstitial fluid as a result of the stasis of lymph, which is caused by an obstruction of the lymph vessels).

Breast: Capsule contractures secondary to breast implants are disorders of fibers and are conditions of fixed high resistance (rigidity) to passive stretch of a muscle. Fibrocystic disease, selected cases of breast cancer where there is tissue loss may also benefit from treatment with elastin peptides.

Cartilage growth: Transformation of hyaline cartilage to elastin cartilage in remaking of structures such as an ear, nose, larynx or any structure in which elastic cartilage would be beneficial, could be aided by treatment with elastin peptides.

Ear: Chronic serous otitis media and hearing loss secondary to otitis media as well as other diseases causing scarring of the ear drum can benefit from replacement of elastin which can serve to repair scarred ear drum tissue caused by these chronic infections.

Eye: Eye disorders such as diabetic retinitis, retinal hemorrhages associated with pseudoxanthoma elasticum (PXE), macular degeneration, and retinitis pigmentosa all involve abnormalities of the retina which is comprised in part of elastic fibers. PXE is an inherited disorder in which elastic fibers become slowly calcified, producing characteristic changes in the skin, the retina of the eye, and the cardiovascular system. Incorporation of healthy, normal elastin peptides to the retinas of individuals affected by these disorders could improve vision and lead to healing of the retina and prevention of further damage caused by the lack of or presence of malformed elastin in this tissue.

Genito-urinary tract: There are various genito-urinary conditions which are associated with either chronic inflammation or other conditions leading to a decrease in elasticity of connective tissue, or with the narrowing of canals or ducts (strictures). The replenishing of elastin or the reversal of the strictures by treatment with elastin and the stimulation of endogenous elastin would benefit a number of genito-urinary conditions including benign prostatic hyperplasia, chronic sclerosing vaginitis, glomerular sclerosis, ureteral stricture, uterine benign fibroids, and vaginal stenosis. Another potential benefit of the present invention in the Genito-urinary tract is the application of the compositions of the present inventions to treat Peyronies disease. Peyronies disease is an induration of the corpora cavernosa of the penis which produces a fibrous chordae. The manifestation of this disease is also known as fibrous cavernitis, penis plastica, and penile induration.

Gastrointestinal tract: A number of gastrointestinal (GI) conditions are the result of chronic inflammation, or abnormal thickening or calcification of GI tissues including anal fissures, chronic pancreatitis, esophageal stenosis, esophageal varices, hemorrhoids, intestinal adhesions, and pyloric stenosis. Crohn's disease as well as other localized inflammatory/fibrotic bowel diseases are characterized by a chronic granulomatous inflammatory condition of unknown etiology. Scarring and thickening of the bowel wall frequently leads to intestinal obstruction and the formation of fistula and abscesses. It is likely that supplying elastin to these tissues may improve gastrointestinal function in these patients and restore the normal balance of connective tissue components in the gastrointestinal tract. Similarly, in biliary cirrhosis and fibrotic liver diseases such as liver

cirrhosis, diffuse and interlacing bands of fibrous tissue form and replace the normal liver lobules.

Immunology: Enhancement of the immune response through cytokine activation as well as suppression of immunity for prevention of transplant rejection and for treatment of autoimmune disorders may be mediated by altering elastin levels. It has been shown that human activated lymphocytes express the elastin-laminin receptor. The expression of the elastin-laminin receptor is a general property of most activated human lymphocytes, but is dependent upon lymphocyte subsets. Elastin peptides activate these receptors and trigger the stimulation of biosynthesis and release of an elastase.

Ulcerations: Ulcers are defects or excavations of the surface of an organ or tissue, produced by the sloughing of inflammatory tissue. Common ulcerative disorders include esophageal, duodenal, and gastric ulcers. It is believed that providing ulcerative tissues with elastin will speed the healing of the affected tissue and possibly even strengthen the tissue by stimulating endogenous elastin production.

Blood Vessels/Heart: Since large amounts of elastin are found in the walls of blood vessels, particularly in the arch of the aorta near the heart, it is important to maintain the normal healthy balance of elastin in blood vessels and other vessels (such as lymph vessels). Additionally, in pulmonary tissues, the subendothelium is comprised of the internal elastic lamina, a layer which normally separates the endothelium from the underlying smooth muscle cells. In many cardiac diseases such as congestive heart failure, coronary artery disease, homocystinuria, restrictive pericarditis, sclerosing endocarditis, supra ventricular aortic stenosis, this internal elastic lamina is compromised due to the breakdown of elastin resulting in a remodeling of this matrix layer. This breakdown is often the result of an imbalance in enzyme(s) (such as elastase) which degrade elastin. In some cases, such as in Marfan's syndrome, the elastin malformations are due to an autosomal dominant, congenital disorder affecting connective tissue. Thus, providing affected tissue with normal elastin peptides may be a useful treatment for strengthening the connective tissue in individuals with Marfan's syndrome.

A bacterial infection caused by the group A beta hemolytic Streptococci resulting in rheumatic fever can sometimes lead to rheumatic heart disease, a serious condition characterized by inflammation and degeneration of connective tissue structures of the body, especially of the heart valves. Treatment of tissues affected by rheumatic heart disease with elastin peptides may allow these tissues to heal and be rebuilt. Additional clinical uses of supplemental elastin peptides include use as arterial stents to enhance internal elastic membrane regeneration in angioplasty procedures.

Hypertension: High arterial blood pressure (generally hypertension) can be the result of multiple and diverse etiologies including congenital heart defects, chronic lung disease, hepatic disorders, and autoimmune disease (particularly scleroderma). Hypertension is often marked by endothelial perturbations as well as abnormalities in the subendothelium. These subendothelial problems are manifested in the breakdown of the internal elastic lamina, often by an enzyme which degrades elastin. This breakdown results in the remodeling or rearrangement of the laminar matrix which may result in chronic hypertension. Correcting the elastin composition of the internal elastic lamina with supplemental elastin peptides would improve this condition and would likely augment the standard treatment which includes elastase inhibiting drugs.

With blood vessels and hypertension, a particularly suitable use of the peptides of the present invention would be along with a stent. Depending on the nature of the stent, the stent may have the therapeutic mixture (e.g., peptide(s) alone or in combination with other therapeutic uses) incorporated in the body of the stent or coated thereon. For incorporation, a biodegradable plastic stent will normally be used which will release the therapeutic agents while supporting the vessel and protecting against restenosis. In the fabrication of the stent, the biodegradable matrix may be formed by any convenient means known in the art. Alternatively, the stent may be coated with the therapeutic mixture, using an adhesive or coating which will allow for controlled release of the therapeutic mixture. The stent may be dipped, sprayed or otherwise coated with a composition containing the NO precursor agent or the therapeutic mixture and a matrix, such as biodegradable polymers, a physiologically acceptable adhesive, proteins, polysaccharides, or the like. By appropriate choice of the material for the stent and/or the coating comprising the therapeutic mixture, a

physiologically active amount of the therapeutic mixture may be maintained at the site of the vascular injury, usually for at least one day and up to a week or more.

In Combination with other Skin Enhancing Agents:

In light of the favorable results observed with the elastin peptide composition and the potential applications, the peptide or peptide-like compositions were considered in conjunction (e.g., stepwise administration) or in combination (e.g., a mixture with other skin enhancing agents). Vitamin C (ascorbic acid) and its derivatives are other compounds which have been topically applied as the active ingredient for the treatment of various skin conditions and which would appear useful in combination with the peptides in SEQ IDs 1-75. U.S. Pat. No. 4,983,382 describes the preparation of stabilized ascorbic acid compositions for topical application. It is well known that ascorbic acid (or Vitamin C as it is synonymously referred to herein) is essential to the maintenance of a healthy and attractive skin appearance in humans. Vitamin C helps to stimulate and regulate the production of collagen in human skin tissue thus retarding the formation of wrinkles and otherwise helping to avoid a prematurely aged look to skin. This, in turn, helps to maintain a healthier and younger looking appearance longer. Vitamin C also acts to help prevent or minimize lipid oxidation and other forms of cellular damage resulting from prolonged exposure to the sun's ultraviolet rays, further counteracting premature aging of the skin. It is believed further still that ascorbic acid helps to inhibit (i) the formation of melanin which leads to skin discoloration during the aging process, and (ii) the release of histamine from cellular membranes believed to be responsible for many allergenic reactions, particularly among individuals having so-called sensitive skin. See also, U.S. Pat. Nos. 5,140,043 and 5,122,536, which are incorporated herein by reference.

Another skin enhancing agent that would appear to be suitable in combination or conjunction with peptide SEQ IDs 1-75 would be salicylic acid. It is known to use salicylic acid for the treatment of acne, see for example, U.S. Pat. Nos. 4,891,227 and 4,891,228, to Thaman et al., the disclosures of which are incorporated herein. Further, salicylic acid has been used for the removal of wart, corns and calluses; for the treatment of psoriasis, seborrheic dermatitis and dandruff; and for the topical treatment of ringworm infection. A listing of commercially available products containing salicylic acid can be found in the Physician's Desk Reference, 45th Edition, 1991, page 323.

However, none of these skin enhancing agents have yet demonstrated the positive results that have been seen with retinoids. Retinoids (e.g., Vitamin A and its derivatives) are substances which are known to have a broad spectrum of biological activity. These substances affect cell growth, differentiation and proliferation. Retinoids affect the differentiation, maintenance, and proliferation of many types of cells whether they are of ectodermal, endodermal or mesodermal origin; whether epithelial, fibroblastic or mesenchymal; or whether they are neoplastic, preneoplastic or non-neoplastic. At present, retinoids have found clinical utility in the treatment of severe cystic acne, psoriasis, and other disorders of keratinization. Possible uses of retinoids are being explored in the prophylaxis and treatment of cancer. For a review of developments in retinoid therapy, see Pawson, B. A. et al, "Retinoids at the Threshold: Their Biological Significance and Therapeutic Potential", *Journal of Medicinal Chemistry* 25:1269-1277 (1982). One aspect of the present invention is the combination of retinoids of the peptide or peptides in the group of SEQ IDs I-75. See, e.g., A.S. Zelikson, *J. Cutaneous Aging Cosmet. Dermatol.*, 1:41-47 (1988); J. S. Weiss, *JAMA*, 259:527-532 (1988); J. Bhawan, *Arch. Dermatol.*, 127:666-672 (1991); and L.H. Kligman, *Connect. Tissue Res.*, 12:139-150 (1984). U.S. Pat. No. 4,603,146 also describes the use of Vitamin A acid for retarding skin aging, and U.S. Pat. No. 4,877,805 describes the use of retinoids generally for the same purpose (the disclosures of which are both incorporated herein by reference).

When considering the use of retinoids in skin care products, it is believed that certain retinoids such as, for example, retinol (Vitamin A alcohol), would be preferred over retinoic acid. This is because retinol is an endogenous compound naturally occurring in the human body and essential for good growth, differentiation of epithelial tissues and reproduction. Retinol is also preferred because it has a much larger safety margin than other retinoids such as retinoic acid. Accordingly, attention has turned toward formulating skin care compositions which contain retinol.

Particularly useful in the present invention is Tretinoin. Tretinoin is a yellow to light orange crystalline powder having a characteristic floral odor. The chemical name for tretinoin is (all E)-3,7 dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid. Tretinoin is also referred to as all- trans-retinoic acid and has a molecular weight of

300.44. Retin A and RENOVA[®] are brands of tretinoin (a short for trans-retinoic acid), a substance related to but distinct from vitamin A. RENOVA[®] differs from Retin A in that it contains a moisturizing cream. Retin A (RENOVA[®]) produces multiple effects in the skin. In particular, it increases the responsiveness of skin cells to epidermal growth factor (EGF), the natural hormone that stimulates skin growth.

Typical strength of topical tretinoin (Retin A, RENOVA[®]) creams is 0.025 - 0.1 percent. One study has found that 0.025 percent Retin A may be as effective as 0.05 or 0.1 percent, but with lower incidence of skin irritation. For people with sensitive skin, 0.025% Retin A (RENOVA[®]) may be the optimal strength. According to the studies, improvement on tretinoin (Retin A, RENOVA[®]) may continue for up to a year of continued use.

Early studies by others indicate that RENOVA[®] or Retin A actually decreases the elasticity of the skin. (See Photodamage by Barbara Gilchrest, published by Blackwell Science (1995)). Contrary to this we have found that in combination with or in conjunction with the peptides or peptide-like compounds of the present invention, an improvement in elasticity is detected when using a tretinoin. A Cutometer (Courage & Khazaka, Germany) is used to quantify skin elasticity. The Cutometer's vacuum probe is placed perpendicular to the skin surface to contact the skin and measure its elasticity. This device then generates data which includes several readings: immediate skin deformation, delayed distention, final deformation, and immediate retraction. The Cutometer SEM 575[®] (available from Courage & Khazaka, Germany) allows relatively objective determination of elasticity of the skin (See Skin & Allergy News 30(2): 18, 1999). For details on the Cutometer and the testing methods cited herein one is directed to the text Bioengineering of the Skin Methods and Instrumentation (1995 Catalog Number 8374, ISBN: 0849383749, as well as the 1998 version which are both hereby incorporated by reference thereto in their entirety).

A number of volunteers were treated with the hydrolyzed elastin peptides (as described herein, SEQ IDs 1-41) at a 2% weight concentration versus a group of volunteers who used Retin A (actually RENOVA[®]) first and then applied the 2% hydrolyzed elastin

product. The RENOVA[®] used in the present invention is a tretinoin emollient cream at a 0.05% weight concentration. It contains the active tretinoin (a retinoid) in an emollient cream base. Tretinoin includes RENOVA[®] at a concentration of 0.05% wt/wt in a water in oil emulsion formulation consisting of light mineral oil, NF, sorbitol solution USP; hydroxyoctacosanyl hydroxystearate; methoxy PEG-22/dodecyl glycol copolymer; PEG-45/dodecyl glycol copolymer; stearoxytrimethylane and stearyl alcohol; dimethicone 50 cs; methylparaben, NF; acetate disodium, USP; quaternium-15; butylated hydroxytoluene, NF; citric acid monohydrate, USP, fragrance; and purified water, USP.

As shown in Fig. 7, the hydrolyzed elastin peptides (SEQ IDs 1-41) in a 2% by weight concentration was applied to various test subjects. Over a two-month period, the R2 was between 24% and 23% increase and the R7 was 31%. Even more dramatic were those results obtained by using a 0.05% by weight RENOVA[®] formulation followed by application of the 2% hydrolyzed elastin peptide cream of the present invention. In these patients the elasciticity as measured by R2 and R7 doubled on average.

Table VII illustrates samplings over 3 separate intervals (separated by approximately one month) with 5 separate patients. But for the subject identified as 45 (bg) there was a consistent and dramatic increase in elasticity. Table VII is shown below:

TABLE VII

| | | 1 Month | | 2 Month | | 3 Month | |
|---|--------------|----------|---------|---------|---------|---------|---------|
| Interval | | 1 Pull | 3 Pulls | 1 Pull | 3 Pulls | 1 Pull | 3 Pulls |
| Subject: 41 (th) Fitz: III Gender F-48 | R2 (Gross) | +55% | +62% | +54% | +64% | +57% | +62% |
| | R5 (Net) | +70% | +72% | +66% | +77% | +55% | +68% |
| | R7 (Portion) | +60% | +64% | +56% | +61% | +49% | +61% |
| Subject: 42 (vb) Fitz: IV Gender: F-54 | R2 (Gross) | +75% | +63% | +76% | +76% | +85% | +68% |
| | | *L Cheek | N/A | +78% | +40%* | +82% | +65% |
| | R5 (Net) | +86% | +81% | +77% | +82% | +86% | +81% |
| | | *L Cheek | N/A | +88% | +10%* | +88% | +81% |

| | | | | | | | |
|---|--------------|------------------|-------------|---------------|----------------|--------------|------------------------------|
| | R7 (Portion) | +76% *L Cheek | +67% N/A | +68% +81% | +76% +29% | +82% +83% | +73% +70% |
| Subject 41 (ce) Fitz: III Gender: F-41 | R2 (Gross) | +68% *L Cheek | +67% N/A | +73% +26% | +67% +17%* | +60% +9% | +69% +4% |
| | R5 (Net) | +71% *L Cheek | +77% N/A | +86% +39%* | +89% +50%* | +74% -18% | +68% -14% |
| | R7 (Portion) | +64% *L Cheek | +64% N/A | +74% +26%* | +72% +39%* | +67% +2% | +58% +2% |
| Subject: 44 (ph) Fitz: II Gender: F-50 | R2 (Gross) | +55% *L Cheek | +65% N/A | +65% | +65% | +42% | +59% |
| | R5 (Net) | +77% *L Cheek | +79% N/A | +81% | +79% | +76% | +84% |
| | R7 (Portion) | +65% *L Cheek | +66% N/A | +53% | +66% | +60% | +71% |
| Subject: 45 (bg) Fitz: III Gender: F-52 | R2 (Gross) | +15% | -23% | +2% | 8% **+16% | +1% | +3% **+22% % **+5% |
| | R5 (Net) | +6% | -28% | +25% | +26% **+42% | +38% | +4% **+25% % **-20% |
| | R7 (Portion) | +6% | -28% | -9% | -16% **+17% | +1% | -22% ***+8% % *-10% |

*2nd Biopsy taken L. Cheek

**Monthly interval variances showed a wide range of + and - readings

Please note, this groups' Cutometer readings represent one and three pulls. One pull = total elasticity; three pulls=fatigue and recovery

Figure 7 and Table VII illustrate the desirable results obtained when using various embodiments of the present invention. All readings on the Cutometer have been taken in Mode 1, which is constant negative pressure: 5 seconds on, 5 seconds off. The pull of

three curves indicates the fatigue and recovery of the skin, while one pull indicates the elasticity of the curve. Group 4 studies include both one and three pulls. $R2$ = gross elasticity. It is represented by Ua/Uf which indicates the total pull and relaxation of the skin under negative pressure. $R5$ = net elasticity. It is represented by Ur/Ue which indicates the perpendicular pull and relaxation of the skin under negative pressure. $R7$ = portion elasticity. It is represented by Ur/Uf and is indicative of the portion of elasticity relative to the entire curve. Based upon the Cutometer readings and the in vivo analysis, it appears that the peptides of the present invention stimulate or simulate elasticity of the tissue.

While the foregoing has been set forth in considerable detail, the sequences are presented for elucidation, and not limitation. Modifications and improvements, including equivalents, of the technology disclosed above which are within the purview and abilities of those in the art are included within the scope of the claims appended hereto. It will be readily apparent to those skilled in the art that numerous modifications, alterations and changes can be made with respect to the specifics of the above description without departing from the inventive concept described herein. For example, the compounds can be administered via many alternative drug delivery vehicles known in the art and the peptides can be derived from digestion of elastin or by amino acid sequencing (either solid state or liquid), as well as from over-expression in a bacterial system. Modification (either chemical or enzymatic) of the basic sequences described herein are also within the purview of the present invention. Therefore the disclosed sequences may be modified to include this residue at either the amino or carboxyl ends of the peptides. The sequences may also be chemically modified to increase their activity (e.g., amidation of the carboxyl terminus portion of a sequence). Computational chemistry can be used to predict structure-function relationship, and compounds thus predicted and synthesized may mimic the structure and function of a particular peptide or peptide-like compound disclosed herein and may be utilized. The peptides may be chemically modified to increase their activity. Accordingly, all such variances should be viewed as being within the scope of the present invention as set forth in the claims below.

WHAT IS CLAIMED IS:

1. A composition useful in treating a condition of mammalian tissue, wherein said composition comprises a peptide or biological equivalent thereof, selected from the group consisting of SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, SEQ ID 65, SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 70, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74, SEQ ID 75.
2. The composition of claim 1, wherein said composition is a cosmetic preparation.
3. The composition of claim 2, wherein said cosmetic preparation is formulated as a topical preparation to be applied to a patient's skin.
4. The composition of claim 3, wherein said topical preparation is selected from the group consisting of an emulsion, lotion, spray, aerosol, powder, ointment, cream and foam.
5. The composition of claim 1, wherein the mammalian tissue being treated is a blood vessel.
6. The composition of claim 1, wherein the composition is useful for treating a condition selected from the group consisting of hypertension, coronary heart disease, arteriosclerosis, angina, coronary thrombosis, chronic obstructive pulmonary disease, and restenosis post angioplasty.
7. The composition of claim 1, wherein said peptide is useful in improving tissue turgor.

8. The composition of claim 1, wherein said composition further comprises a pharmaceutically acceptable carrier.
9. A method of enhancing tissue elasticity, said method comprises administering a therapeutically effective concentration of a peptide or biological equivalent thereof, selected from the group consisting of SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, and SEQ ID 65, SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 70, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74, SEQ ID 75.
10. The method of claim 9, wherein the peptide is SEQ ID 55.
11. The method of claim 9, wherein the peptide is SEQ ID 60.
12. The method of claim 9, wherein the tissue is selected from the group consisting of a blood vessel, lung tissue, and skin.
13. The method of claim 9, wherein administration of the peptide is repeated over a predetermined time period.
14. The method of claim 9, wherein the peptide is an active ingredient in a cosmetic formulation.
15. A composition comprising:
 - a skin enhancing agent;
 - and a peptide,
 - said peptide being selected from the group consisting of: SEQ ID 1, SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 5, SEQ ID 6, SEQ ID 7, SEQ ID 8, SEQ ID 9, SEQ ID 10, SEQ ID 11, SEQ ID 12, SEQ ID 13, SEQ ID 14, SEQ ID 15, SEQ ID 16,

SEQ ID 17, SEQ ID 18, SEQ ID 19, SEQ ID 20, SEQ ID 21, SEQ ID 22, SEQ ID 23, SEQ ID 24, SEQ ID 25, SEQ ID 26, SEQ ID 27, SEQ ID 28, SEQ ID 29, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39, SEQ ID 40, SEQ ID 41, SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, SEQ ID 65, SEQ ID 66, SEQ ID 67, SEQ ID 68, SEQ ID 69, SEQ ID 75, SEQ ID 71, SEQ ID 72, SEQ ID 73, SEQ ID 74, SEQ ID 75, their biological equivalents and combinations of any of SEQ IDs 1-75.

16. The composition of claim 15, wherein said skin enhancing agent is a retinoid.
17. The composition of claim 16, wherein said retinoid is all-trans retinoic acid.
18. The composition of claim 16, wherein said retinoid is Retin A.
19. The composition of claim 16, wherein said retinoid is tretinoin.
20. The composition of claim 15, wherein said peptide is SEQ ID 17.
21. The composition of claim 15, wherein said peptide is SEQ ID 19.
22. The composition of claim 15, wherein said peptide is selected from the group consisting of SEQ ID 1, SEQ ID 2, SEQ ID 3, SEQ ID 4, SEQ ID 5, SEQ ID 6, SEQ ID 7, SEQ ID 8, SEQ ID 9, SEQ ID 10, SEQ ID 11, SEQ ID 12, SEQ ID 13, SEQ ID 14, SEQ ID 15, SEQ ID 16, SEQ ID 17, SEQ ID 18, SEQ ID 19, SEQ ID 20, SEQ ID 21, SEQ ID 22, SEQ ID 23, SEQ ID 24, SEQ ID 25, SEQ ID 26, SEQ ID 27, SEQ ID 28, SEQ ID 29, SEQ ID 30, SEQ ID 31, SEQ ID 32, SEQ ID 33, SEQ ID 34, SEQ ID 35, SEQ ID 36, SEQ ID 37, SEQ ID 38, SEQ ID 39, SEQ ID 40, SEQ ID 41.
23. A peptide having a formula of R₁-Valyl-Valyl-Prolyl-R₂, wherein R₁ is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10

amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

24. The peptide of claim 23, wherein the peptide is selected from the group consisting of SEQ ID 15, SEQ ID 42, SEQ ID 43, SEQ ID 44, SEQ ID 45, SEQ ID 46, SEQ ID 47, SEQ ID 48, SEQ ID 49, SEQ ID 50, SEQ ID 51, SEQ ID 52, SEQ ID 53, SEQ ID 54, SEQ ID 55, SEQ ID 56, SEQ ID 57, SEQ ID 58, SEQ ID 59, SEQ ID 60, SEQ ID 61, SEQ ID 62, SEQ ID 63, SEQ ID 64, and SEQ ID 65.

25. The peptide of claim 23, wherein the peptide is SEQ ID 17.

26. The peptide of claim 23, wherein R₂ is selected from the group consisting of a glutamine residue and modified glutamine residue.

27. A peptide having a formula of R₁-Valine-Valine-Proline-Asparagine-R₂, wherein R₁ is an amino portion modified to include an amine, amide, or amino acid sequence having 1-10 amino acids and R₂ is a carboxy portion modified to include an amide, ester, or carboxy terminus sequence having 1-10 amino acids.

28. The peptide of claim 27, wherein the peptide is SEQ ID 55.

29. The peptide of claim 27, wherein the peptide is SEQ ID 60.

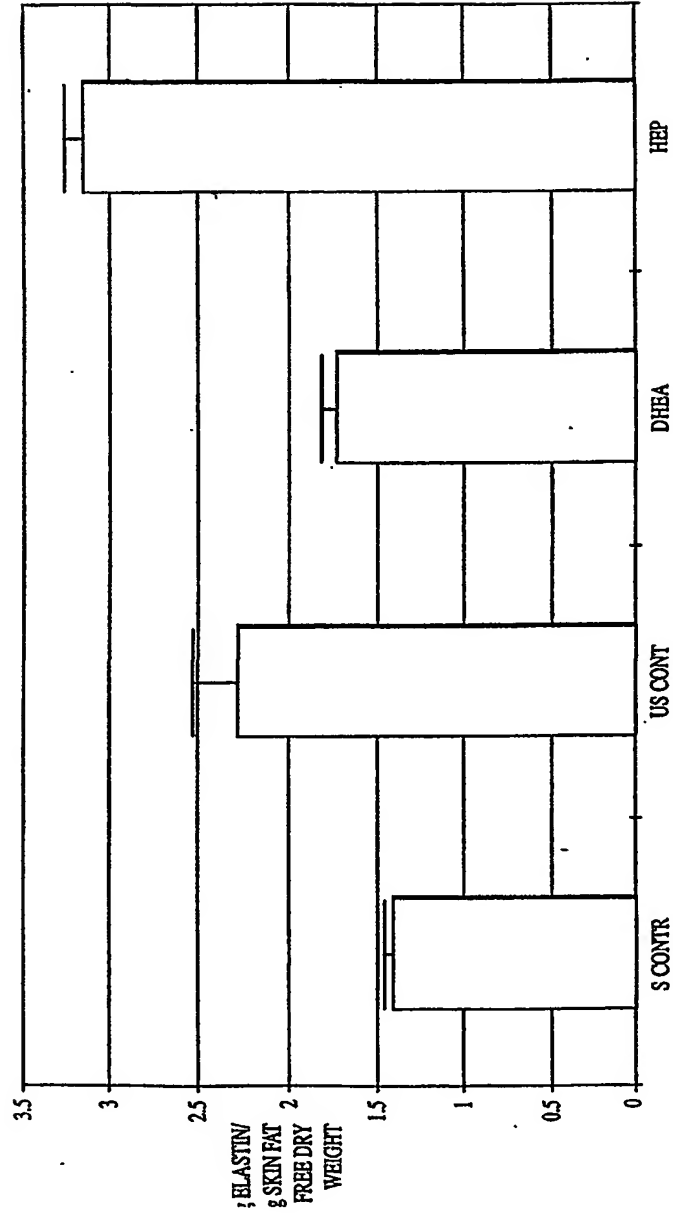
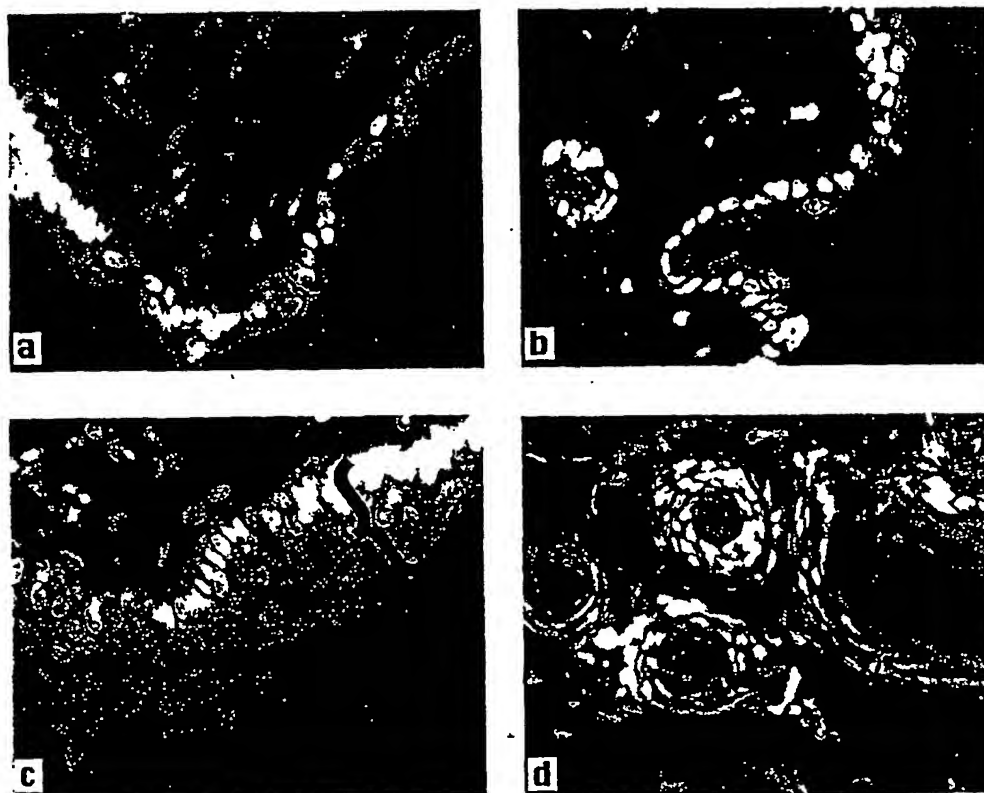
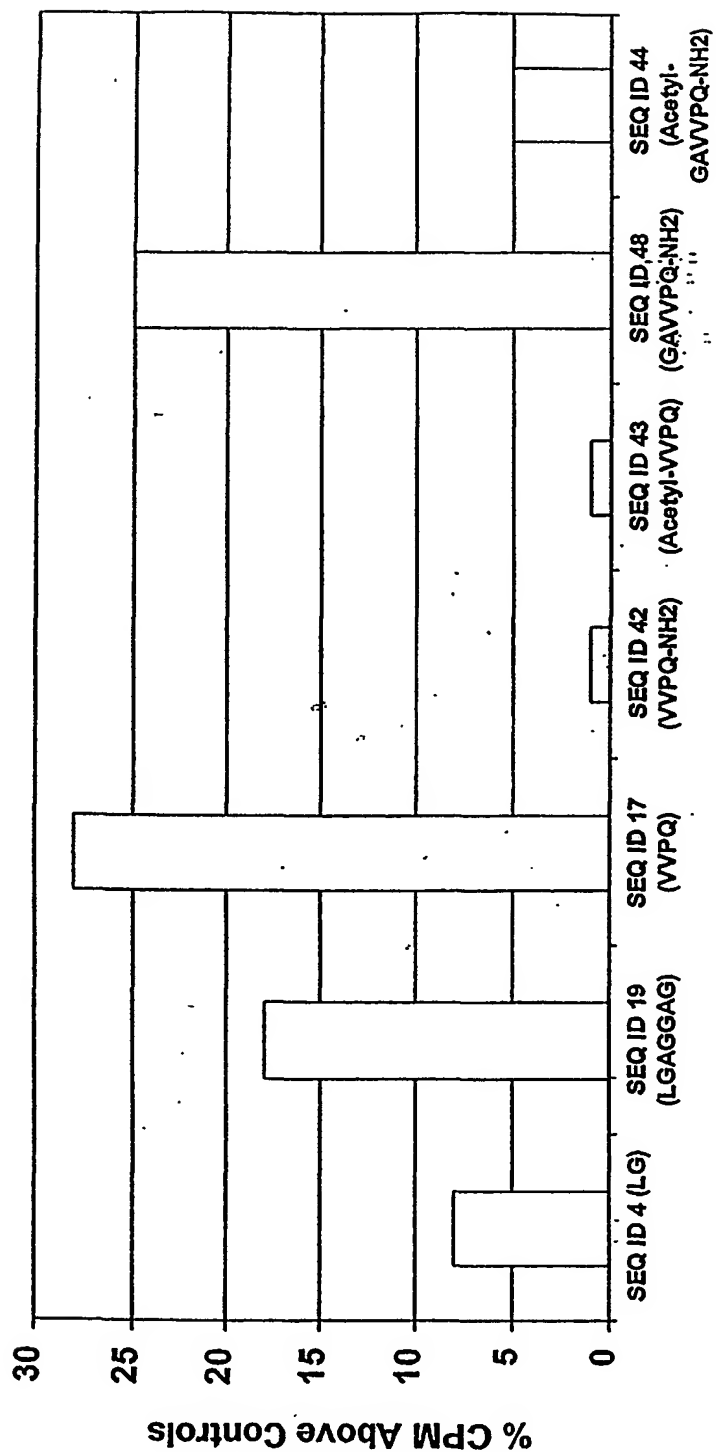


FIG. 1

FIG. 2



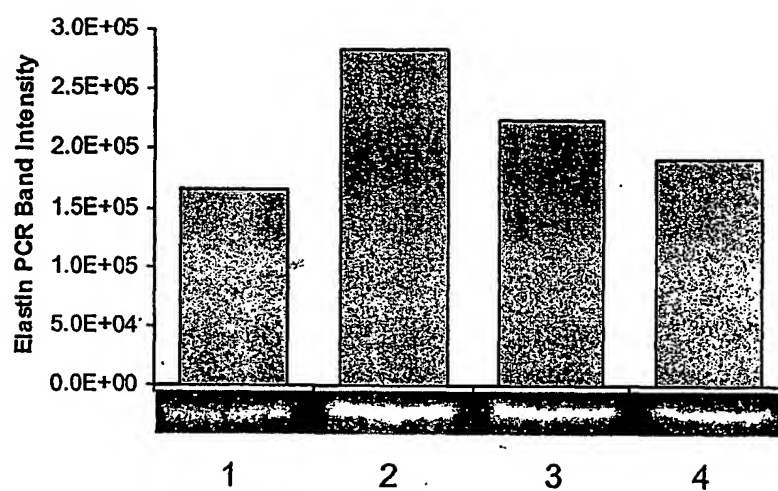
Tritiated Thymidine Incorporation with a 24 hr. Incubation of RFL-6 Cells



Synthetic Peptides (Including VVPQ and Analogues of VVPQ)

FIG. 3

FIG. 4



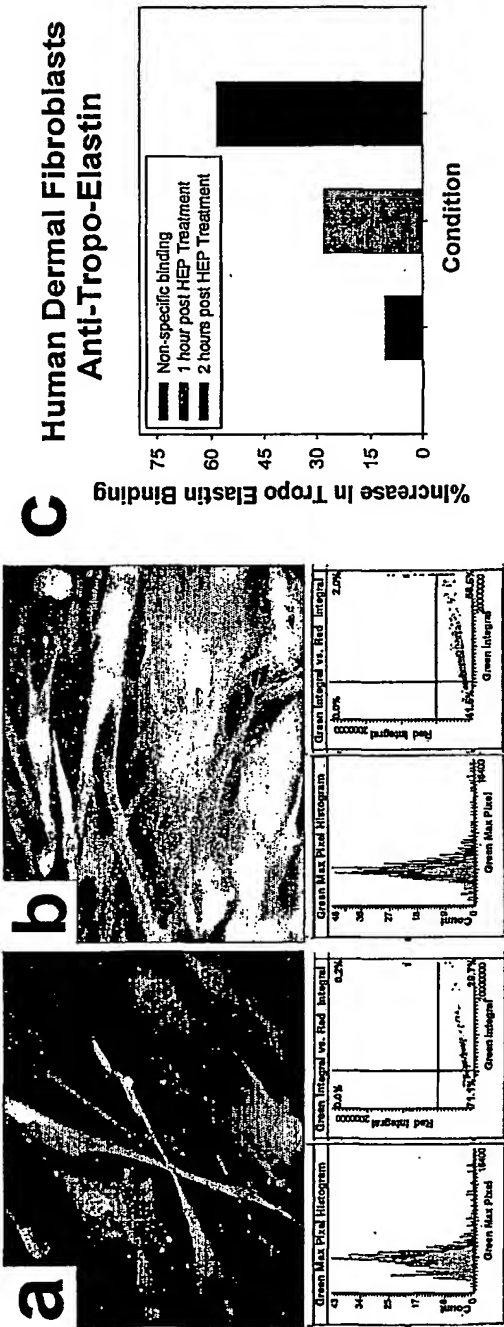
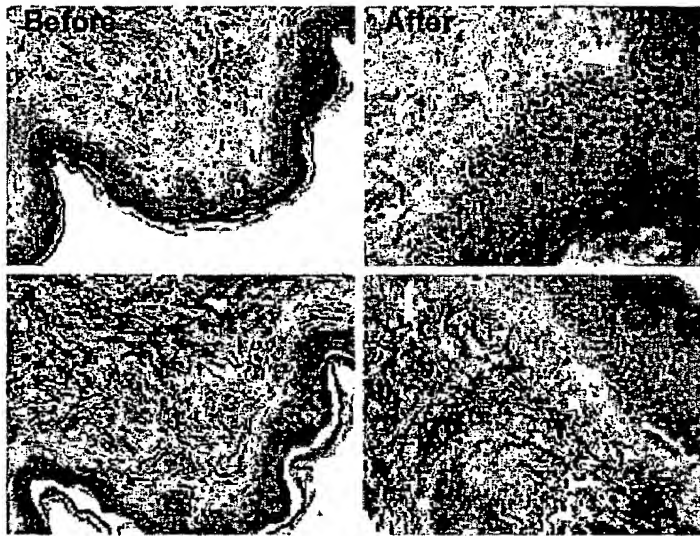


FIG. 5

FIG. 6



2 Month Study of HEP vs. (HEP and RENOVA)

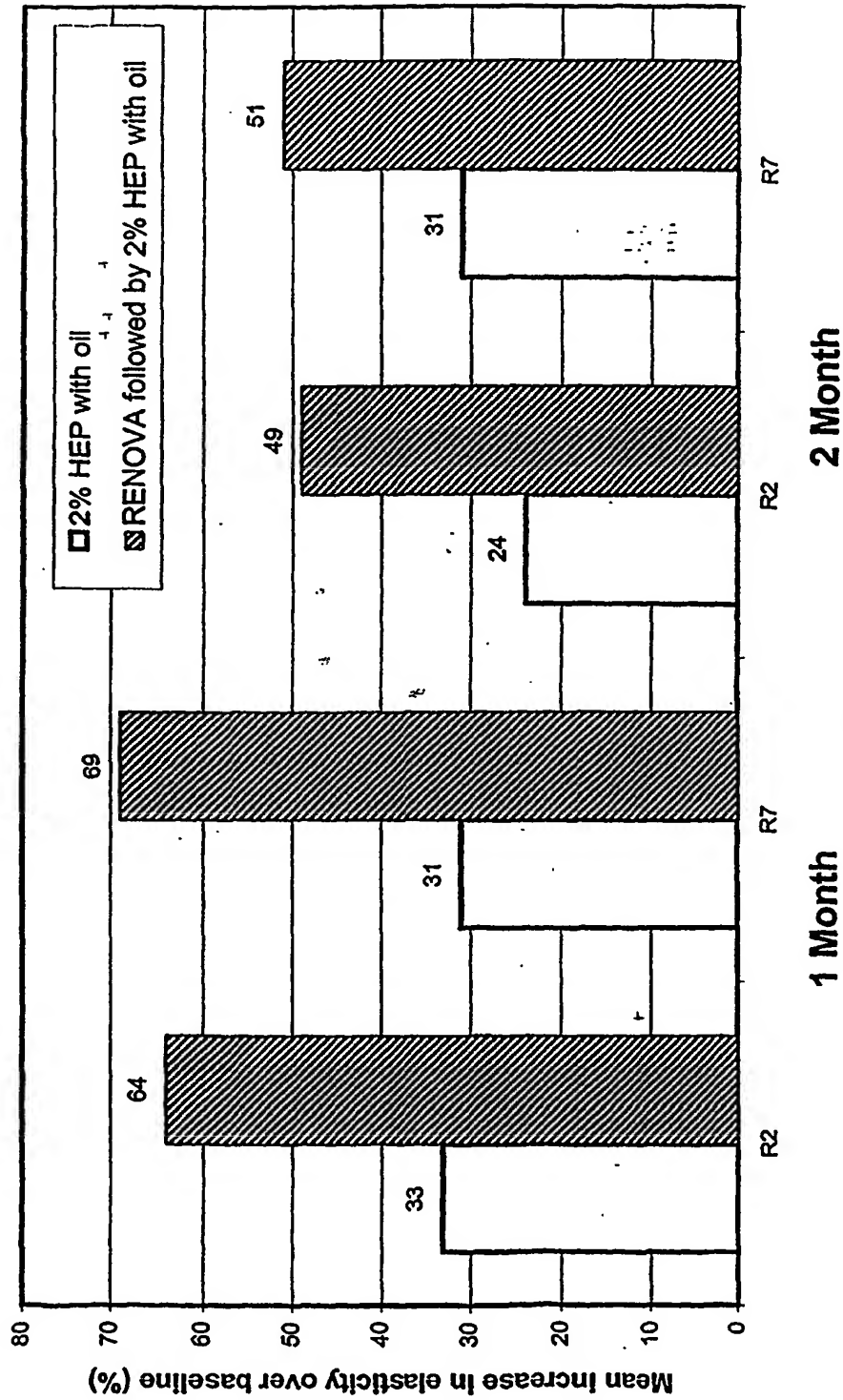


FIG. 7

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